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I. Summary. /for non-specialists/.

Our knowledge of the physiology of the pituitary gland is mainly based upon experiments on laboratory animals, especially on rats. Moreover the regulation of secretion and release of gonadotrophic hormones controlling sexual cycles and lactation is not known. In experiments on laboratory animals the hypothalamus has been proved to be the regulating organ of the pituitary secretion. Namely, when the pituitary gland in rats and ferrets is separated from the hypothalamus, whether by division of the pituitary stalk or by transplantation of the gland away from the sella turcica, follicular activity in the ovaries ceases. The situation in the literature with regard to the lactotrophic hormone /LTH, prolactin, controlling the function of corpus luteum and lactation/ is quite different, for secretion of this hormone, after Desolin and Everett, continues after transplantation of the hypophysis. Our observations, however, made in rabbits and dogs seem to show that the hypothalamus also in secretion of LTH plays a controlling role; namely, the rabbits and dogs /bitches/ with pituitary stalk section /performed in the second period of pregnancy/ gave normal litters but they were unable to nurse them because of the lack of milk secretion. It is thought that the hypothalamus influences the pituitary gonadotrophic function by neurosecretory material/s/ /neurohormones/ carried from median eminence /lower part of the hypothalamus/ to the adeno-hypophysis by the vascular way, namely, through the so called portal vessels of the pituitary stalk. But up to date any substance of such an ability has not been known. Discrepancies and unclear phenomena such as those set above and especially the lack of experiments in this matter on farm animals show the need to undertake investigations on such problems in farm animals. We started our experiments on sheep. The sheep in spite of its domestication have retained their natural behaviour at the onset of seasonal cycles, and for this reason it is very suitable as an experimental animal for neuroendocrinological studies in the physiology of reproduction of farm animals. In the first year of investigations we have worked on:

- /i/ recognition of anatomical vascular relations between hypothalamus and hypophysis in sheep.
- /ii/ elaboration of surgical technique of the pituitary stalk section in sheep.
- /iii/ preliminary experiments on searching for substance/s/ in hypothalamus responsible for the release of pituitary gonadotrophins.

ad/i/ The blood supply and the so called portal network system of the stalk and hypophysis show some anatomical variability in particular species of mammals. References on this problem in sheep are very scanty. In our experiments, therefore, we had to start with the recognition of the anatomical vascular relations. We maintain there is main blood supply drained to the adeno-hypophysis by the hypophysial portal vessels in the pituitary stalk. However, from the capsule of the pituitary gland penetrate into the periphery of the adeno-hypophysis several very tiny twigs which probably may supply the blood in the event of blocking of the

main blood circulation /through the stalk/. This recognition will enable us to go ahead on our experiments and to draw in the future proper conclusions from experiments.

- ad /ii/ Due to the shape of the cranial skull of sheep and, connected with this, great difficulties in performing the pituitary stalk section after the classical method by the trans-temporal approach, we have elaborated a new method of the pituitary stalk section in sheep. Our method has the advantage in comparison to the classical one, owing to its simplicity and shorter period of healing of the wound after the operation. Using this method there were 10 sheep operated on and 8 animals survived. The surviving animals will be used to further experiments in 1962.
- ad /iii/ In preliminary experiments on searching for substance/s/ in the hypothalamus responsible for the release of pituitary gonadotrophins the attempts were made by "in vitro" incubation of adenohypophysis slices with hypothalamus tissue fragments. The tissues were incubated 24 hours in a special solution /Earle solution/ at 38°C. After incubation, the gonadotrophins were extracted from the incubating medium and tested by bioassay on virgin mice by measuring the growth rate of the uterus under influence of injected extracts. It was found that the addition of median eminence tissue to incubated pituitary slices increased distinctly the release of pituitary gonadotrophins. The investigations therefore seem to prove the presence in the median eminence of cattle of some unidentified substance/s/ stimulating even under artificial conditions the release of gonadotrophins.

II. Detailed Report /for specialists/.

Introduction.

When the pituitary gland is separated from the hypothalamus whether by division of the pituitary stalk or by transplantation of the gland away from the sella turcica, follicular activity in the ovaries ceases /1,2/. Investigations with lesions placed in some parts of the hypothalamus and especially in eminentia mediana give also evidence of hypothalamic control of gonadal function, particularly of ovulation. The experiments with lesions, conducted by various authors, however, differ concerning their results and interpretation. In experiments of Clegg and Ganong /3/ destruction of the ventral hypothalamus in sheep was associated with absence of both ovarian and behavioral cycles, and the destruction of an area immediately above the anterior median eminence inhibited oestrous behaviour without affecting the ovarian cycles. While in experiments of Donovan and van der Werff ten Bosch in ferrets /4,5/ it was found that, when electrolytic lesions were placed in the anterior hypothalamus of anoestrus, sexually quiescent animals, there was rapid activation of the gonads and the animals became oestrous long before blank - operated controls. All the above cited experiments are not quite compatible with one another and not yet

elucidating the way of hypothalamo - hypophysial regulations. However, they have proven that the secretion of FDH /follicle developing hormone/ by the hypophysis is controlled by the stimulating or inhibiting action of the hypothalamus, exerted very likely by neurosecretory material/s/ carried from median eminence to the adenohypophysis on vascular way, namely, through the portal vessels of the pituitary stalk.

The situation in the literature with regard to the luteotrophic hormone /LTH/ is quite different, for the secretion of this hormone, after Deselin /6/ and Everett /7/, continues after transplantation of the hypophysis. Grafts also of pituitary tissue have been shown to release LTH when inserted into otherwise intact mice /8/.

Our observations, however, made in rabbits and dogs seem to show that the hypothalamus also in secretion of LTH plays a controlling role, namely, the rabbits and dogs /bitches/ with pituitary stalk section /performed in the second period of pregnancy/ gave normal litters, but they were unable to nurse them because of the lack of milk secretion. Discrepancies such as those set above and the lack of experiments on this matter with farm animals gave rise to undertaking investigations on such problems in farm animals. The sheep, in spite of its domestication, retained its natural behaviour at their onset of sexual cycles, and for this reason it seems to be very suitable as an experimental animal for neuroendocrinological studies in the physiology of reproduction of farm animals.

2. The plan of investigations to be undertaken on the problem.

1-st year.

- /i/ recognition of anatomical vascular relations between hypothalamus and hypophysis in sheep.
- /ii/ elaboration of the surgical technique of the pituitary stalk section in sheep and surgical preparation of 10 animals /with pituitary stalk section/ for further experiments.
- /iii/ Preliminary experiments on searching for substance/s/ in the hypothalamus responsible for the release of pituitary gonadotrophins.

3. Realization of the 1st year's plan.

- /i/ Anatomical vascular relations between hypothalamus and hypophysis in sheep.

The blood supply and the so-called portal network system of the stalk and hypophysis show some anatomical variability in particular species of mammals. The references to this problem in sheep are very scanty. Daniel and Richard /9/ are of the opinion that in goats and sheep there is only one way of arterial supply to the anterior pituitary and this way is the hypophysial portal system of the stalk.

The stalk section, therefore, after the above cited authors, is followed by an immediate extensive infarction and necrosis of the anterior pituitary.

Our investigations of the vascular relations between the hypothalamus and hypophysis were performed by injection of indian ink into the carotid artery intra vitam and also by injection under pressure of indian ink into the carotid artery of the dead animal /after slaughter/. It has been shown in these injected preparations that the main blood supply to the hypophysis in sheep flows through the so-called hypophysial portal vessels in the stalk. There is, however, another possibility of arterial blood supply to the hypophysis. Namely, the internal carotid arteries running along the fossa pituitaria give off a multitude of small arterial branches building a great arterial plexus around the pituitary gland. From this plexus a multitude of small branches penetrate into the capsule and into the posterior lobe of the pituitary. To the adenohypophysis, however, only several very tiny twigs penetrate from the capsule into the periphery of the gland. It is suggested therefore that this blood supply to the adenohypophysis is responsible for surviving of the periphery of the adenohypophysis and its regeneration after stalk section, stated by Daniel and Richard /12/. These authors stated namely, that with the passage of time after the pituitary stalk section the surviving cells around the periphery of the adenohypophysis regenerate and grow into the area which had been infarcted.

In respect of such a findings we wonder whether anterior hypophysis deprived of direct neurohumoral pathway - would be able or not to retain its gonadotrophic activity.

/ii/ Elaboration of surgical technique of the pituitary stalk section in sheep.

The shape of the cranial skull of sheep and especially its great narrowness and depth make the operation of pituitary stalk section after the classical method by the transtemporal approach very difficult and nearly impossible.

In spite of that in the transtemporal approach in sheep the upper cranium /upper parts of ossa temporales/ is opened as wide as possible and the brain is turned upside down, the access to the operation site is very troublesome. In our experiments, therefore, we have elaborated a new method of the pituitary stalk section. In this method we use special instruments. The instrument for the stalk section /its operative part/ has the form of a flat arc edged on the end and on one side only; its concave surface is adapted to the shape of the brain in the region of the central and lateral fissures. The second instrument is the forceps for inserting an impenetrable barrier between the cut ends. This forceps has the analogical shape as that of the instrument to the stalk section; moreover the forceps has a special arrangement for pushing down the plaxiglass plate.

After having opened the cranium in the form of an quadrangular window /20 x 30 mm/ in the area at the right orbital margin, the instrument for the section is introduced between the cortex and the dura and led down half-way stereotactically between the chiasma and the pituitary stalk. The reaching of the operation site is evidenced by the length of the introduced instrument and by feeling the opposite border of the pituitary fossa. After having reached the operation site, the stalk is severed by small movements of the instrument in the direction opposite to the chiasma. The insertion of the plaxiglass plate between the cut ends is performed in an analogical half stereotaxic way as in the stalk section. The sheep's head during the operation must be tightly fixed; to this purpose special apparatus has been constructed. Our method has an advantage in comparison to the classical one owing to its simplicity in technique and shorter period of time in healing of the wound after the operation.

Up to the 1st of January 1962 with this method there were 10 sheep operated on and 8 animals survived.

The surviving animals will serve as experimental animals for further investigations.

/iii/ Preliminary experiments on searching for substance/s/ in hypothalamus stimulating the release of pituitary gonadotrophins.

At the present stage of our investigations on the pituitary gonadotrophic function we are searching for active substances in the hypothalamus responsible for the release of gonadotrophins. These attempts are made in two ways, by "in vitro" incubation of adenohipophysis slices with hypothalamic tissue fragments, and by injection of hypothalamic extracts into experimental animals.

The investigation "in vitro" is comprised of a 24 hour incubation of fine slices of adenohipophyses of cows with median eminence tissue fragments /H/ of the same animals. The tissues were incubated in 100 ml of Earle solution in incubating flasks at 38°C (40).

As a control, incubation of pituitary sections alone /K/ and with cortex cerebri tissue from opthalmic lobus /M/ were also performed. After 24 hours, the gonadotrophins were extracted from the incubating medium using Klinefelter and Brandbury method, modified by Loraine and others. For bioassay of each series 12 virgin mice were used. The extracts in saline were injected into suitable mice groups, once daily during 3 days. The animals were killed after 72 hours. The uteri were dissected and weighed. The increase in uterus weight was considered as an indirect test for gonadotrophins content in the incubating medium. Generally 10 complete series of investigations were performed. The incubation of pituitaries alone /K/ induced the release of gonadotrophins to the incubating solution. The uterus showed a 1,9 fold

increase in weight in comparison to that of the controls. Incubation of pituitaries with cortex cerebri /M/ had no influence of the increase of gonadotrophins quantity in the extract. The uterus weight in this group was similar to that of the former one /K/. The addition of median eminence tissue to incubated pituitary slices /H/, however, increased distinctly the release of pituitary gonadotrophins. The increase of uterus weight, in comparison to that of the control group, was 2,7 fold and to that of group /K/ 1,4 fold.

The investigations therefore seem to prove the presence in the median eminence of cattle of some unidentified substance stimulating even under artificial conditions the release of pituitary gonadotrophins.

To investigate the effect of the hypothalamic extract on the pituitary gonadotrophic function "in vitro" 10 rabbits were prepared by the surgical method. The arteria carotis was translocated directly under the skin. Thus a so-called "skin bridge" for arteria carotis was formed. By this way, it will be possible to inject hypothalamic extracts into the carotid artery and to introduce biological or chemical substances directly into the central nervous system by passing the main blood circulation. Rabbits, operated on in such a way will serve as test animals in searching for active substance/s/ in the hypothalamus responsible for the pituitary gonadotrophic function. The rabbit is a very suitable test animal for that purpose owing to its property of induced ovulation.

Plan for the 2 - n d year 's i n v e s t i - g a t i o n s .

- /i/ Observations of sexual cycles and other symptoms in animals after the pituitary stalk section.
- /ii/ Experiments on searching for substance/s/ in hypothalamus responsible for the release of pituitary gonadotrophins.
- /iii/ Technical and methodical preparation for neurophysiological investigations upon some neural centers in hypothalamus controlling the secretion of FDH and LTH.

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